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Photodynamic activity of porfyrinic derivatives front candida albicans.

Atividade fotodinâmica de derivados porfirínicos frente candida albicans.

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RESUMO

O uso inadequado de antifúngicos tem causado resistência da Candida aos tratamentos convencionais, dificultando o controle da candidíase oral. Isso demanda a busca por alternativas. A terapia fotodinâmica (TFD) é estudada como método para inativar microrganismos patogênicos no hospedeiro. Ela combina fotossensibilizadores com luz e oxigênio, gerando espécies citotóxicas que atuam na membrana celular desses microrganismos. Um estudo avaliou a atividade fotodinâmica de compostos porfirínicos em isolados de Candida albicans. Foram testados cinco isolados clínicos e uma cepa ATCC de Candida albicans, tratados com dois agentes fotossensibilizantes derivados de porfirinas, 3-PtTPyP e 4-PtTPyP, associados a radiação laser vermelha de 30 J/cm2. Após o tratamento, as colônias foram contadas em ágar Sabouraud após 48 horas de incubação a 30°C. Os resultados, expressos em UFC/mL, foram analisados com Graphpad Prism® 5.0. As cepas estudadas foram sensíveis à inativação fotodinâmica, especialmente à 3-PtTPyP, sugerindo-a como uma possível opção terapêutica contra infecções por Candida albicans.

Palavras-chave: Terapia fotodinâmica. Candida albicans. Laser. Compostos Porfirínicos.

ABSTRACT

The inappropriate use of antifungals has led to Candida's resistance to standard treatments, complicating the control of oral candidiasis and prompting the quest for alternative therapies. Photodynamic therapy (PDT) has been explored to deactivate pathogenic microorganisms within the host. This method combines a photosensitizer with light and oxygen, generating cytotoxic species that target the cell membrane of these microorganisms. This study aimed to assess the photodynamic activity of porphyrinic compounds in Candida albicans isolates. Five clinical isolates and one ATCC strain of Candida albicans underwent photodynamic treatment using two photosensitizing agents, 3-PtTPyP and 4-PtTPyP, at a concentration of 0.1 mg/mL, coupled with red laser radiation of 30 J/cm2. Post-treatment, aliquots were cultured on Sabouraud agar, and colonies were counted after 48 hours of incubation at 30°C, with results expressed in colony-forming units per mL (CFU/mL). The CFU/mL data from control and test groups were analyzed using Graphpad Prism® 5.0 (Graphpad Software, INC). The strains exhibited sensitivity to photodynamic inactivation, particularly with the porphyrin 3-PtTPyP, suggesting its potential as a therapeutic option for Candida albicans-associated infections.

Keywords: Photodynamic Therapy. Candida albicans. Laser. Porphyrinic Compounds

1. INTRODUÇÃO

In the oral environment there are more than 500 species of microorganisms in the buccal environment, usually commensal, but which in certain circumstances can have pathogenic potential[1]. The genus Candida is in the oral cavity as part of the microbiota and is isolated in about 60% of healthy individuals, but can pathogenetically compromise with hosts regardless of their immune status.[2,3]. The species Candida albicans is prevalent and causes about 60% of the infections leading to the occurrence of candidoses [1].

The treatment for oral candidiasis of healthy patients is usually of local use, with prescription of topical antifungal agents. Among the topical antifungal agents, the most commonly used are miconazole and nystatin, which can cause side effects such as hypersensitivity and problems in the gastrointestinal tract. Amphotericin B and azole derivatives are the most common systemic antifungal agents. Itraconazole and fluconazole are the azole derivatives of the oral suspension of first choice, since they are safer and more effective [1]. The indiscriminate use of antifungal drugs as well as the high occurrence of recurrent candidiasis in immunocompromised patients makes it possible to develop resistant strains. Fluconazole is the drug of choice for the treatment of candidiasis in HIV patients. However, it was verified that 23% of Candida strains isolated from these patients were resistant to this antifungal [3].

In view of this, it became necessary to study methods, such as Photodynamic Therapy (PDT) that, alternatively, can fight the microorganisms that cause the candidoses [4]. PDT is used for the treatment of malignant neoplasms and diseases caused by fungi, viruses and bacteria. This therapy works by associating a laser light and a photosensitizer in the presence of oxygen, which once takes the photophysics inside the cell with the formation of reactive oxygen species (ROS). Photosensitizers, usually dyes such as methylene blue, toluidine blue and porphyrin derivatives, when associated with light and at the appropriate wavelength, have an antimicrobial effect [5]. This mechanism of PDT causes the death of the microorganisms from the inviability of their cells [4]. For the photodynamic therapy to perform better, a photosensitizer with specific photochemical and photophysical characteristics should be used, such as selectivity by target cells, good absorption in the region of 600 to 1000 nm, high quantum yield and good biological response [6].

Porphyrin derivatives are photosensitizers widely used in photodynamic therapy due to intense optical absorption, high chemical and photochemical stability, and high affinity with biological structures [7,8]. It's fundamental the study and knowledge of new techniques

that facilitate the professional to be successful in the treatment of oral infections in the dental practice. he development of new therapies such as PDT can provide an adequate treatment without side effects and that allows to optimize the conventional antifungal treatment already worn by resistance of the pathogenic microbiota of the host to the medication. Testing the effectiveness of new therapies and making them a possible option over existing methods will contribute significantly to the patient and the dentist.

The objective of this study was to evaluate the antifungal potential of in vitro photodynamic therapy, associated with porphyrin macrocycles (3-PtTPyP and 4-PtTPyP) and laser InGAIP, 30 J / cm2, against Candida albicans isolates.

2. MATERIAIS E MÉTODOS

This In vitro study was carried out at the Laboratory of Mycological Research (LAPEMI) of the Federal University of Santa Maria, Santa Maria, Rio Grande do Sul. The microorganisms used were five clinical isolates and an ATCC 14057 strain of Candida albicans from the Laboratory of Mycological Research (LAPEMI) of the Federal University of Santa Maria. These isolates were stored in distilled water with 10% glycerol at -20ºC and were identified by classical methods and molecular methods. The experiments were carried out with porphyrin derivatives, synthesized in the Chemistry Department of the Federal University of Santa Maria. To obtain the synthetic porphyrins meso-tetra-[(4-pyridyl) porphyrinato] chloro platinum (II) and meso-tetra-[(3-pyridyl) porphyrinato] chloro platinum (II) (4-PtTPyP e 3-PtTPyP). It used the methodology of Rothemund[9], followed by the reaction of metallation of the porphyrin ring with their respective metal salts.

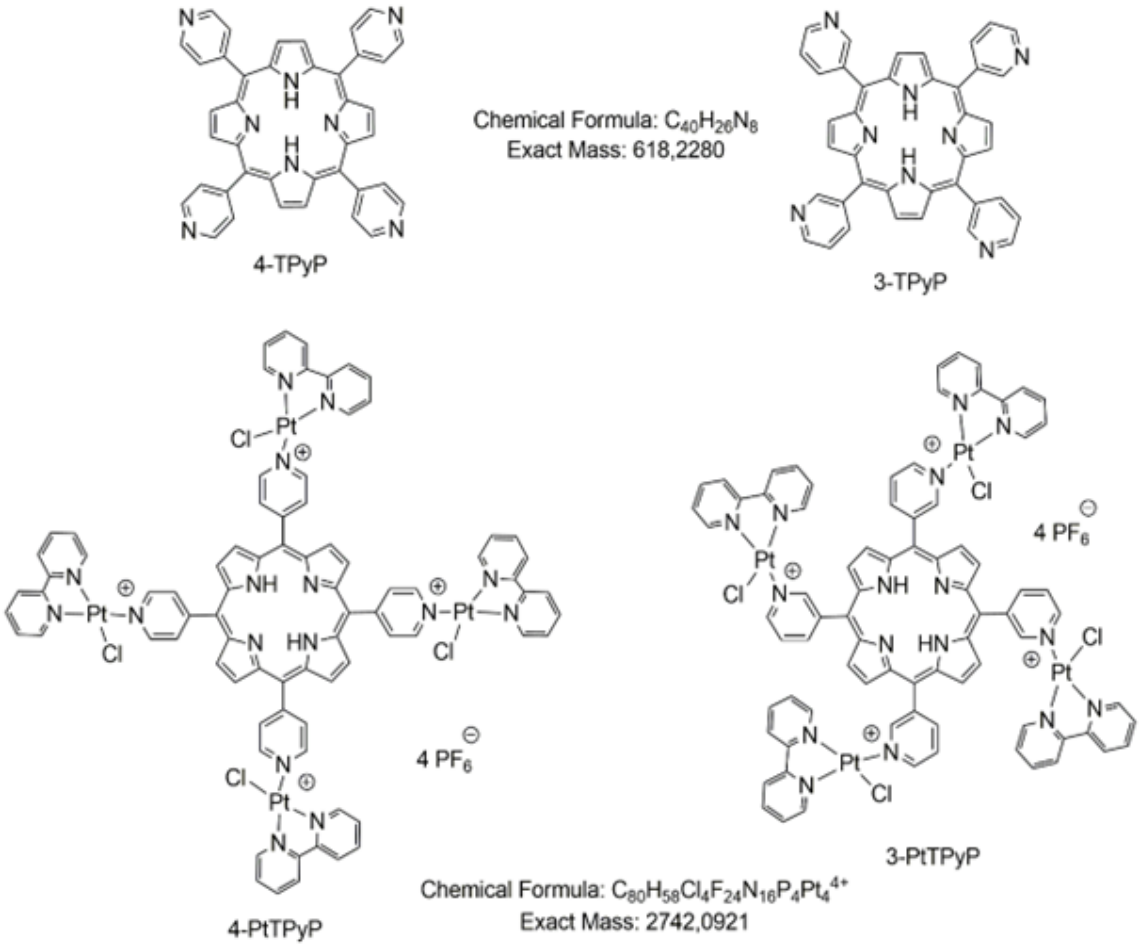

Figure 1: Structural representation of 3-PtTPyP and 4-PtTPyP porphyrins

As light source, a diode laser (Na-GaAlP; Theralase, DMC, São Carlos, Brazil) was used with a power of up to 100 mW at a wavelength of 685 nm and at a distance of 1 cm from the surface of a microplate with an irradiation of up to 100 mW / cm², which didn't produce any photothermal effect from 300 mW /cm² and induced an increase of 0,5ºC in solution after 2 min of red light irradiation. Thus, there was no need to control the temperature to avoid heating effects.

To prepare the inoculum subcultures of C. albicans, isolates were performed on Sabouraud dextrose agar, incubated at 30°C for 48 hours. The colonies were then suspended in 0,85% sterile saline solution. The resulting suspension was vortexed for 15 seconds and the adjusted turbidity equaled to 0,5 tube of the McFarland scale ($1x10^6$ at $5x10^6$ cells per ml). The photodynamic treatment was performed based on the methodology described by Lyon et al. (2013)[10] with some modifications. Compounds were tested at the final concentration of 0,1 mg/mL. Volumes of 100 µL of each concentration were added to 100 µl of standardized fungal inoculum and then dispensed into wells of sterile 96-well

plates.

The suspension was incubated for 30 minutes at 30°C and protected from light. After that period, the inoculums were irradiated using the diode laser (InGaAIP, Theralase, DMC, São Carlos, Brazil), with a useful power of 35 mW at 685 nm wavelength, at a distance of 1 cm for 1 minute and 50 seconds. The irradiated area was 0,38 cm², resulting in an energy dose of 28 J/cm². The following controls were included: fungal inoculum without irradiation or photosensitizer (C1); fungal inoculum exposed to 0,1mg / mL 3-PtTPyP without irradiation (C2a); fungal inoculum exposed to 0,1mg / mL of 4-PtTPyP without irradiation (C2b); isolates exposed to irradiation without photosensitizer (C3). The tests were composed of fungal inoculum with 3-PtTPyP (Ta) or 4-PtTPyP (Tb) and irradiation. After the irradiation period, 50 ul of the solution was diluted in saline (1:100) and aliquots of 10 µL of each group were inoculated on Sabouraud dextrose agar and incubated at 30°C for 48 hours for counting the colonies. All tests were performed in duplicate. The photodynamic activity was evaluated by comparing the non-irradiated and free photosensitizer control (C1).

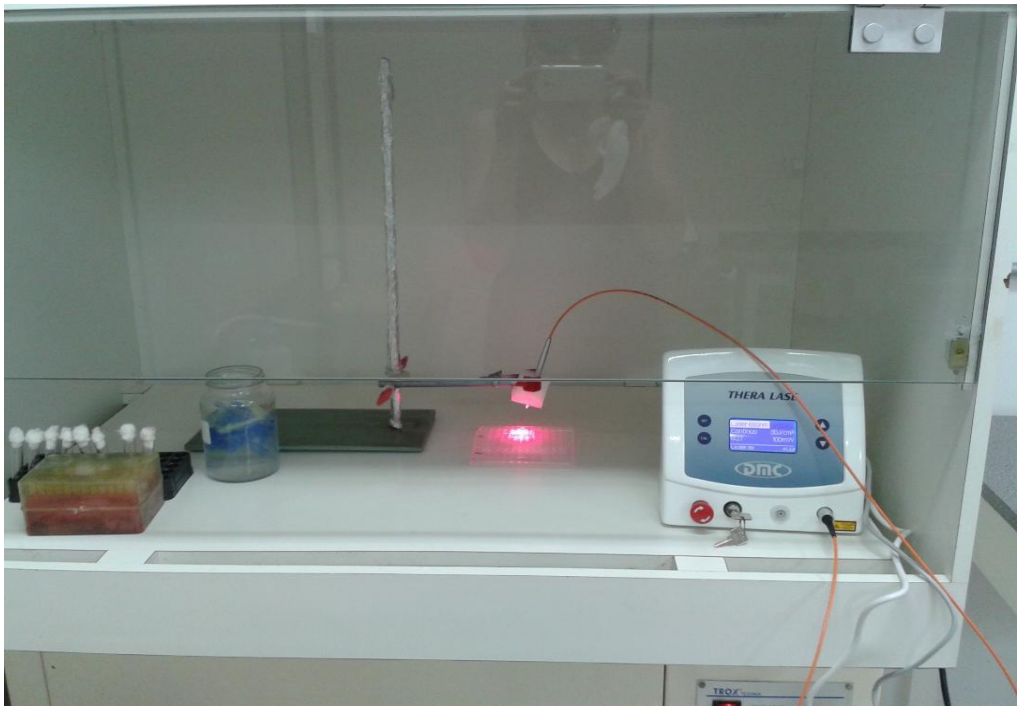

Figure 2: Photodynamic treatment - Laser irradiation in 96 well plates**.**

The data were analyzed using Graphpad Prism® software 5.0 (Graphpad Software, INC). The Mann-Whitney test was used to compare the control group (C1) with the test groups, considering a significant difference when $p < 0.05$.

3. RESULTADOS

After treatment of the five clinical isolates and strain ATCC 14057 and statistical analysis, it can be seen, according to Table 1, that the treatment with 3-PtTPyP and 4-PtTPyP porphyrins was effective, since there was a significant reduction ($p < 0.05$) in CFU / mL in relation to the control group (C1). In addition, it was possible to observe that the treatment performed with porphyrin 3-PtTPyP was more satisfactory, since in four of the six treated species, when compared to 4-PtTPyP, the total number of CFU / mL was lower in relation to group C1.

Table 1: Effect of photodynamic therapy with 3-PtTPyP or 4-PtTPyP and led (InGaAlP,100 mW / cm2) against Candida albicans isolates

	UFC/mL					
Isolados	C1	C2 (3-PtTPyP)	C2 (4-PtTPyP)	C3	T (3-PtTPyP)	T (4-PtTPyP)
ATCC 14057	$8,48x10^5$	$3,1x10^5$	$5,4x10^4$	$5,29x10^5$	***4,7x10^3**	**3,7x10^4**
Ca21	$1,73x10^5$	$2,7x10^4$	$1,78x10^5$	$1,52x10^4$	***Zero**	***4,9x10^4**
Ca38	$2,56x10^5$	$2,4x10^4$	$5,8x10^4$	$3,0x10^5$	***1,05x10^2**	***2,4x10^3**
Ca44	$6,58x10^5$	$2,01x10^4$	$1,4x10^4$	$1,58x10^5$	***4,05x10^2**	***Zero**

Ca45	$1,3x10^6$	$6,05x10^3$	$4,37x10^5$	$1,33x10^6$	***Zero**	***$9,3x10^4$**
Ca49	$1,22x10^6$	$1,37x10^5$	$3,28x10^5$	$6,57x10^5$	***$9,0x10^3$**	***$9,3x10^4$**

C1 growth in Sabouraud Agar Dextrose without irradiation or 3-PtTPyP / 4-PtTPyP; C2 (3-PtTPyP) isolates exposed to 0.1 mg / mL 3-PtTPyP without irradiation; C2 (4-PtTPyP), isolates exposed to 0.1mg / mL of 4-PtTPyP without irradiation; C3, isolated exposed to irradiation without 3-PtTPyP / 4-PtTPyP; T (3-PtTPyP), photodynamic treatment with 0.1 mg / mL 3-PtTPyP; T (4-PtTPyP), photodynamic treatment with 0.1 mg / mL 4-PtTPyP. *** p<0,05**

4. DISCUSSÃO

The present study evaluated the possible antifungal potential of PDT in vitro using synthetic porphyrins meso-tetra - [(4-pyridyl) porphyrinato] chloro platinum (II) and meso-tetra - [(3-pyridyl) porphyrinato] chloro platinum (II) in Candida albicans isolates. The results showed a significant reduction ($p < 0.05$) in the treated groups compared to controls containing only inoculum (C1) and there was no growth of Candida albicans in three of the twelve tests performed. It can also be verified that, despite the same molecular formula as 4-PtTPyP porphyrin, 3-PtTPyP porphyrin was more efficient.

Several researchers have used a growing number of photosensitizing agents and light sources to inactivate Candida species for decades 11,12,13,14,15,16,17,5. The activity of these FSs is related to their chemical and photochemical properties, as well as the cellular structure of the pathogens that will be treated18. Porphyrins, like FSs, have a high potential for use in PDT, since their agents are photosensitive to wavelengths in the range of 630 nm, in addition to the cytoplasmic properties when activated by the light source 19. Carmello et al. (2016)20 used porphyrin-derived FS-Photodithazine® at concentrations of 150 and 175 mg/L to sensitize Candida species with the LED device at 660 nm and an energy dose of 37,5 J/cm², following methodology similar to this study. The authors used biofilms of Candida

albicans, Candida glabrata and Candida tropicallis, obtaining a great reduction in the growth of Candida species studied (p <0.05), results similar to the present study with the use of planktonic cells. Quiroga et al. (2010)21 obtained complete inactivation of C. albicans, planktonic cells and biofilms, using porphyrin TMPyP, indicating the potential of this FS in antimicrobial therapy, as well as the porphyrin derivative of the present research, since the reduction of growth of all treated species was satisfactory (p <0.05).

In vivo study Carnello et al. (2016)22 obtained equivalent CFU/mL reduction values from the previous in vitro study from Candida isolates obtained from the dorsum of the mouse tongue using the porphyrin derivative Photogem. Mima et al. (2009)23 also demonstrated the effectiveness of porphyrin derivatives in vivo, using PDT with the same FS from the previous author in the tongue of mice. The CFU/mL reduction of the tests in relation to the control was significant (p <0,01). These studies make feasible as well as the in vitro studies of biofilms and planktonic cells of Candida albicans and other species, the use of porphyrin agents in humans.

The realization of the present study with PDT seeks to meet the need to establish alternatives for the control of microorganisms resistant to treatment with traditional antibiotics, through the photodynamic therapy associated with porphyritic macrocycles, due to their great versatility and functionality of generation of reactive oxygen species. Alves et al. (2017)24 demonstrated that PDT mediated by FS Photodithazine reduced (p <0.05) the susceptible and fluconazole resistant biofilm of Candida albicans. A similar study by Mang, Mikulski, Sala (2010)25 evaluated the effect of Photofrin PDT on Candida ATCC strains and on resistant and sensitive isolates fluconazole and amphotericin B from samples from AIDS patients. ATCC isolates and patient isolates showed sensitivity to PDT. Such studies show that PDT and the use of porphyrin derivatives are an alternative treatment for patients with resistance to conventional antifungals.

In addition to the use of PDT alone, its combination with conventional treatment can generate synergistic results, decreasing dosages of the drug and photosensitizing agent and also reducing treatment time In a study by Quiroga et al. (2016)26 the association of PDT-mediated porphyrins TAPP and TAPP4 + with fluconazole resulted in antifungal action was more effective than the two treatments isolated against C. albicans in vitro. Davies et al. (2016)27 obtained similar results with the porphyrin derivative TMP-1363 against biofilms of C. albicans, C. glabrata, C. tropicalis and C. parapsilosis. The combination of PDT associated with miconazole was additive in relation to the isolated therapies.

It is possible to analyze that the results of the use of porphyrin macrocycles in this

study are as effective as the use of other photosensitizers. Hoisseini et al. (2016)28 evaluated four photosensitizers in the impossibility of biofilms of Candida albicans and suspensions of Candida dubliniensis and Candida albicans. Methylene blue, aniline blue, malachite green and violet crystal were tested and the results indicated reduction of CFU/mL of the tests in relation to the control groups ($p < 0,05$) and there was no statistical difference between the photosensitizers studied. Silva et al. (2017)29 made possible the use of PDT in Candida kruzei, an opportunistic pathogen that presents intrinsic resistance to fluconazole. The use of PDT associated with toluidine blue inhibited both growth and biofilm formation by the microorganism.

In addition to the feasibility of using PDT in the fight against Candida, Nemézio et al. (2017)30 and Nagal et al. (2017)31 obtained in vitro bactericidal effect of S. mutans from the use of low power lasers associated with methylene blue. The results found in these researches allow the use of PDT in the treatment of oral diseases, not only fungal but of bacterial origin, such as dental caries and periodontal disease.

The results evidenced in previous research corroborate with the results of the present study, since different porphyrin derivatives, as well as other photosensitizers, have been shown to be effective in the control of Candida spp. species such as Candida albicans. Therefore, this photosensitive agent is a treatment option for diseases caused by these and other microorganisms both in the medical and dental fields. It is important to note that 3-PtTPyP and 4-PtTPyP porphyrins should be subjected to further laboratory studies, especially with a larger sample and spectrum of pathogens tested. In addition, the importance of establishing more comparisons with the photosensitizers traditionally used in compadrios vitro research and clinical photodynamic treatments.

5. CONSIDERAÇÕES FINAIS

Based on the results obtained in this study, it was possible to conclude that the photodynamic therapy, using the porphyrinic macrocycles 3-PtTPyP and 4-PtTPyP was effective against the studied strains. In addition, it can be observed that 3-PtTPyP porphyrin appears to have had more significant activity when compared to 4-PtTPyP, this agent being a possible therapeutic option of photosensitizer against infections associated with Candida albicans.

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